

DOMINANCE HIERARCHY FORMATION IN JUVENILE CRAYFISH *PROCAMBARUS CLARKII*

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Summary

The formation of social dominance hierarchies was studied in groups of five juvenile crayfish, 1.3–1.8 cm in length. Animals were grouped together in a small, featureless aquarium after having lived in isolation for more than a month. The occurrence of each of four behavior patterns ('attack', 'approach', 'retreat' and 'escape') was recorded for each animal, together with the frequency of encounters and the frequency of wins and losses. The frequencies of wins and losses were used to calculate the relative dominance value of each animal in the group. High levels of fighting developed immediately upon grouping the animals, and a positive feedback relationship between attacking and winning enabled one animal in each group to emerge quickly as the superdominant. If that animal was the largest, it remained as the superdominant; otherwise, it was replaced as superdominant within the first

few days by the largest animal. This form of dominance hierarchy, with one superdominant and four subordinates, persisted throughout the duration of the grouping. Fighting declined over the first hour and by 24 h had dropped to low levels. After the first day, approaches were used together with attacks, and retreats replaced escapes. Attack and approach were the behavior patterns displayed most frequently by animals with high dominance values, whereas retreat and escape were performed by animals of low dominance. All these trends continued to develop over the next 2 weeks as the number of agonistic encounters declined to a low level.

Key words: dominance, hierarchy, tailflip, escape, retreat, attack, behaviour, crayfish, *Procambarus clarkii*.

Introduction

Within a population of social animals, conflict will arise between unacquainted animals over the division of a valuable resource that is in limited supply (Pusey and Packer, 1997; Moynihan, 1998). This conflict leads to the formation of a social dominance hierarchy that enables peaceful, if uneven, divisions of resources to occur whenever the resources are contested. Conflict can also occur between unacquainted animals when tangible resources are absent. This conflict, which has been seen to be a dispute over social dominance (or future access to resources) (Clutton-Brock and Parker, 1995), also leads to hierarchy formation.

These patterns are followed in crayfish populations, in which agonistic interactions between unfamiliar pairs of crayfish lead to hierarchy formation both when tangible resources are in dispute and when they are not. For example, larger crayfish evict smaller conspecifics from burrows that are closer to food sources (Ranta and Linström, 1992), and groups of unacquainted crayfish fight and form dominance hierarchies when no resource other than space is contested (Bovbjerg, 1953; Lowe, 1956). As the first example suggests, a size difference is the major determining factor in these contests

(Pavey and Fielder, 1996; Ranta and Linström, 1992; Bovbjerg, 1953), although experience (Pavey and Fielder, 1996), maternal state (Figler et al., 1995b) and residence (Peeke et al., 1995; Ranta and Linström, 1992) can also play significant roles. If the animals are dissimilar in size, the smaller of the two may give way without a fight or the fight may be very brief. If they are similar in size, however, fights may escalate before one animal signals defeat by withdrawing (Bruski and Dunham, 1987; Huber and Kravitz, 1995). Subsequent to that decision, the interactions between such a pair will become much more peaceful as the subordinate avoids the dominant and refuses to contest access to resources (Copp, 1986).

In small groups of crayfish, these pairwise interactions lead to the formation of dominance hierarchies (Copp, 1986; Ranta and Linström, 1992; Bovbjerg, 1953). The rankings within these hierarchies form a linear sequence that stabilizes after the initial set of contests. Less is known about how the hierarchical relationship forms over time and is subsequently maintained. Specifically, we do not yet know how the behavior patterns of individuals change during that formation and how these

behavior patterns both lead to, and depend on, changes in relative dominance. The present study addresses these questions for groups of five juvenile crayfish with no prior social experience that were observed over a 2 week period during the formation of their social hierarchies.

Materials and methods

All crayfish used in this study were juvenile *Procambarus clarkii* (Girard) collected 1 week after hatching, as each dropped from its mother's swimmerets and became free-swimming. They were then raised in individual containers for 1 month until they attained a size suitable for study. This procedure minimized the animals' prior social experience at the time of grouping. The animals in each group were of the same sex, and were similar in age and molt status (the 'C' molting stage) (Huner and Avault, 1976). They spanned a range of sizes between 1.3 cm and 1.8 cm measured from rostrum to telson. No differences between groups based on sex were apparent.

From a total of 32 groups of five crayfish, five groups experienced fewer than two fatalities over the 2–4 week period of observation; data from these animals are presented here. Animals were measured and marked for identification the day before formation of the group. Each group was formed when five juveniles were taken from isolation and placed simultaneously in a 10 cm×13 cm×18 cm observation aquarium. The five animals in each group were labeled alphabetically in order of size with 'A' as the largest. The aquarium was filled with fresh water to a depth of 5 cm and was lined with 2 cm of fine black gravel to aid in contrast and traction. No objects were available that could provide shelter. The group was formed at dusk, when crayfish are most active (Page and Larimer, 1972). Their behavior was immediately videotaped for 1 h. They were again filmed for a 1 h period at the same time on day 2, and at the same time at intervals of between 1 and 3 days for up to 30 days, depending on the group. Each group was observed at least once during each 3 day period following day 2 until day 14. To compare the responses of the different groups over the first 2 weeks of observation (see Fig. 3), average responses over each 3 day period were calculated for each group. The animals were maintained in the observation aquarium for the rest of the experiment under a 12h:12h light:dark cycle, and were fed brine shrimp every other day after filming. The water was kept at 20 °C and aerated constantly except during videography. All five animals survived for 13 days in four of the five groups; in one group, one animal died after 6 days.

Analysis and evaluation

Analysis of the interactions among animals in a group was conducted from examination of the video recordings. All interactions involving agonistic behavior were termed *encounters*. Each encounter was recorded as a chronological sequence of four easily distinguishable agonistic behavior patterns, 'attack', 'approach', 'retreat' and 'escape'. In our

usage, an attack is an aggressive physical contact initiated by one animal on another. An attacking animal approaches its target quickly, as in a charge, and grabs it with the chelipeds. An approach is a slower movement of one animal towards another that does not lead to contact but does evoke a response (i.e. one of the four behavior patterns) from the other. A retreat is an ambulatory movement away from an approaching or attacking animal. An escape is a rapid movement away from an aggressor produced by one or more tailflips (i.e. rapid flexions of the abdomen). Other agonistic behavior patterns (e.g. an offensive tailflip in which one animal drags another by tailflipping) occurred rarely, and only on the first day, while still other non-agonistic interactions (in which one animal crawls over another) were noted but excluded from this study. This categorization of agonistic behavior patterns approximates those of Bovbjerg (1953) and Lowe (1956).

One simple encounter between crayfish A and B might have been recorded as follows: A attacks B from the front; B retreats backwards. A more complex encounter might include as many as 20 individual actions listed sequentially. An encounter was considered over when the animals became separated by more than two body lengths and their behavior became uncorrelated. The loser of an encounter was identified as the animal that broke off the encounter and moved more than two body lengths away from the other animal, which was then considered the winner.

Determination of cardinal dominance values

To assign a daily and quantitative relative dominance value to each juvenile crayfish within a group, we followed the model of Boyd and Silk (1983). The cardinal dominance values were calculated by arranging the numbers of wins scored by each animal against those of each of the other animals in a winner/loser matrix, and then applying an iterative algorithm to the matrix (Boyd and Silk, 1983). The dominance value calculated for each animal in the group depends on all the values in the winner/loser matrix. The Boyd and Silk method assumes (i) that the outcome of an encounter is probabilistically independent of the outcomes of earlier encounters, (ii) that dominance relationships between any three animals in a group are stochastically transitive (if animal A is likely to beat animal B, and B is likely to beat C, then A is more likely to beat C) and (iii) that the number of encounters between any pair is independent of their respective dominance ranks.

Assumption i requires that the hierarchy be in a steady state during the period of observation. This is problematic during the first hour of observation when the initial hierarchy is established, but should be less so on subsequent days. Unfortunately, the small numbers of encounters after day 1 do not allow cardinal dominance calculations over fractions of an hour, so that a trend over an hour could not be determined. Assumption iii is also problematic during the first hour when most of the encounters resulted from attacks made by one animal on the others. Because the attacking animal usually won, a strong correlation exists between the number of

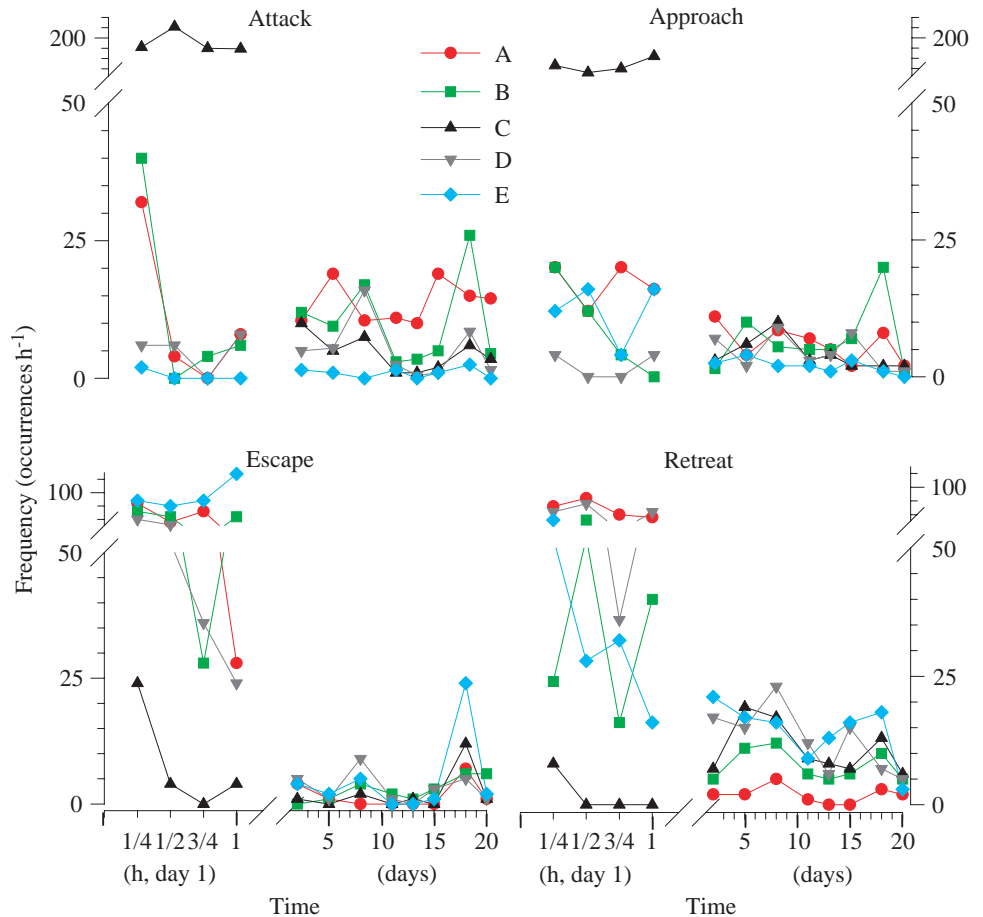


Fig. 1. Behavior patterns of individuals in group 1 during hierarchy formation. The frequencies of four different types of behavior (attack, approach, escape and retreat) of individual animals (A–E, labeled from largest to smallest) are plotted over the 20 day period of observation. Each point on day 1 (to the left of the break on the time axis) represents the frequency (occurrences h^{-1}) over a 15 min period; points for later days each represent the frequency for a 1 h observation period on that day. Data collection occurred at intervals of between 1 and 3 days (see Materials and methods). In the upper row of plots ('Attack' and 'Approach'), the small divisions above the break along the abscissa represent frequency intervals of $40 h^{-1}$; in the lower row of plots ('Escape' and 'Retreat'), they represent $20 h^{-1}$.

encounters between pairs of animals and the difference in their cardinal dominance values. This correlation does not exist for subsequent days when the number of attacks was more evenly distributed among the animals of a group. Despite the violations of the assumptions, the cardinal dominance values calculated for the first day are qualitatively consistent with the observation that one animal dominates all the others.

Cardinal dominance values cannot be calculated when one animal failed to interact with one of the other animals during the observation period, when one animal won or lost all its encounters or when dominance relationships were circular, violating assumption ii above. Where possible, cardinal dominance values were calculated for 15 min intervals during the first hour of interaction on the first day, and for each hour of observation on subsequent days. In groups 4 and 5, however, one animal failed to lose while another failed to win during some of the 15 min periods of observation on the first day. In these cases, dominance values were calculated for one 30 min period instead of two 15 min periods.

Results

Behavior of individuals in a group: immediate onset of fighting on the first day

Fighting broke out among the five animals of each group

immediately upon formation of the group and remained intense throughout the first hour of interaction. One animal in each group quickly emerged as the most active, engaging in more fights and winning more victories than the others. In group 1, animal C, the mid-sized animal in the group, constantly moved from animal to animal, attacking or approaching each, engaging in 250 encounters over the hour (Fig. 1). These attacks were very brief, usually lasting less than 1 s, but were often so vigorous that even the largest animal, A, escaped from C at the first physical contact.

The four less-active animals in group 1 moved about the arena less vigorously and occasionally interacted with each other between encounters with animal C. The few approaches they made were directed at each other. The attacks of C and approaches by the other four evoked frequent retreats and escapes from those four. The largest animal (A) made more retreats than escapes, whereas the reverse was true for the smallest animal (E).

In four of the five groups, an initial high frequency of attacks and approaches by one animal effectively arrested attacks and minimized approaches by the other four animals. In those four groups, the most active animal maintained a high level of attacks throughout the hour, whereas the other four animals attacked infrequently throughout the hour (three groups) or began with frequent attacks that grew infrequent over the hour

(two groups) (Fig. 2A). A result of these changes is that the difference between the number of attacks made by the most active animal and the sum of attacks made by the other four animals increased at the beginning of the hour, achieved a peak and then declined slightly over the last part of the hour (Fig. 2B). A similar pattern was observed in the wins achieved by the most active animal and the wins achieved by the other four animals (Fig. 2C,D).

Decline in the frequency of agonistic behavior

The frequency of encounters in group 1 dropped from a peak of nearly 400h^{-1} during the second 15 min of observation on day 1 to 65h^{-1} on day 2 (Fig. 3A). A similar decline in activity also characterized the other four groups of crayfish. Among all groups, the frequency of encounters fell over the first hour from an average of 300h^{-1} to less than 200h^{-1} , and then to an average of 50h^{-1} in all groups on day 2 (Fig. 3B). Agonistic activity continued to decline in frequency over the next few days, although much more slowly. This decline is apparent in the average over all groups, which fell to less than 30h^{-1} by day 14.

Change in the frequency of different types of behavior

In addition to a general decline in the frequency of agonistic interactions, the relative frequencies of different behavior patterns also changed. In group 1, there were twice as many attacks as approaches by all animals during the first 30 min of

day 1, but by the end of the hour the frequency of attacks had fallen to equal that of approaches (Fig. 3A). On subsequent days, their frequencies remained approximately equal, with a slightly higher proportion of attacks than approaches (Fig. 3C).

The reduction in the frequency of attacks in group 1 after the first day was coupled with a large decrease in the frequency of escapes. The proportion of encounters in which escapes occurred fell dramatically, from more than 90% of encounters on day 1 to 20% on day 2 (Fig. 3C). This decline was common to all animals in the group; the frequency of escape of each animal from each of the others was reduced to a low level after the first day. In contrast, the overall frequency of retreats remained high; in group 1, as the number of escapes declined, the proportion of retreats increased from 50% to more than 80% (Fig. 3C). As a result, retreats became the preferred means of withdrawal over escapes by a margin of nearly 4:1 on day 2. Retreats were performed by animals in approximate inverse order of size: E, D and C retreated more frequently than B, and B retreated more frequently than the newly aggressive A (Fig. 1). The preference for retreat over escape persisted during the rest of the period of observation of group 1.

A similar pattern of change was seen in all the other groups. The mean frequency of each type of behavior across all five groups is plotted for all four behavior patterns in Fig. 3B. The frequencies of all types of behavior experienced a gradual decline over the first hour, an initial sharp decline between days

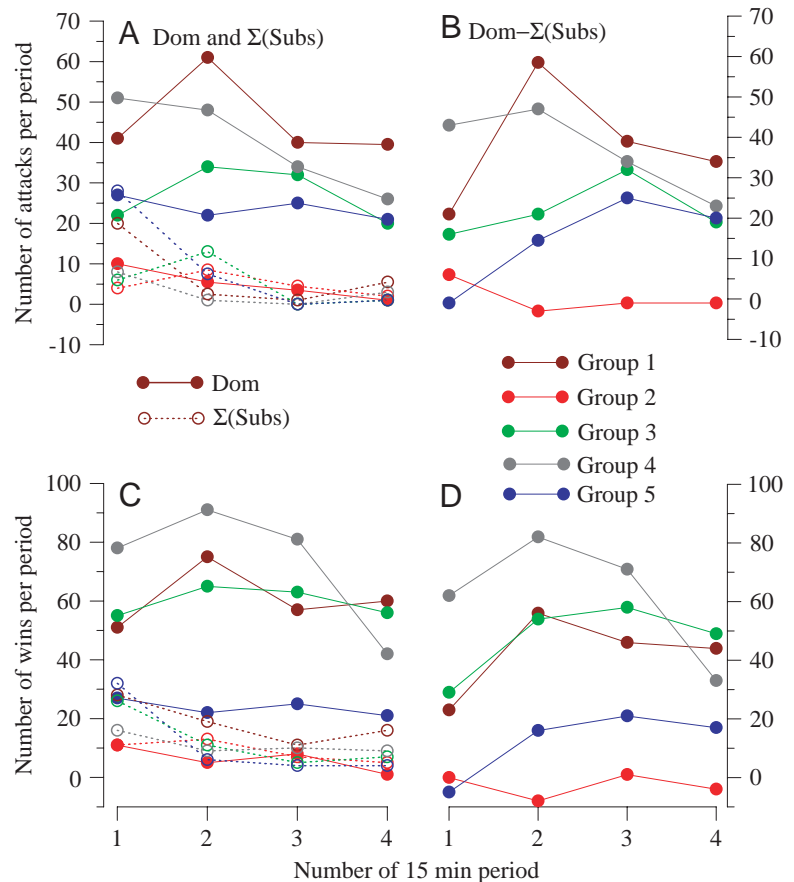


Fig. 2. Rates of attacks and wins in all five groups during the hour of observation on the first day. (A) Rates of attack made by the most dominant animal (Dom: filled circles and unbroken lines) in each group, and the sum of the rates of attack made by the other four animals in that group [$\Sigma(\text{Subs})$: open circles, broken lines] for each 15 min period of observation on the first day. (B) The difference between the rate of attack of the most dominant and the sum of the rates of the other four animals in each group [$\text{Dom}-\Sigma(\text{Subs})$]. (C) The rates of winning of the most dominant animal (filled circles and unbroken lines) in each group, and the sum of the rates of winning of the other four animals in that group (open circles, broken lines). (D) The difference between the rate of winning of the most dominant animal in each group and the sum of the rates of winning of the other four animals in that group.

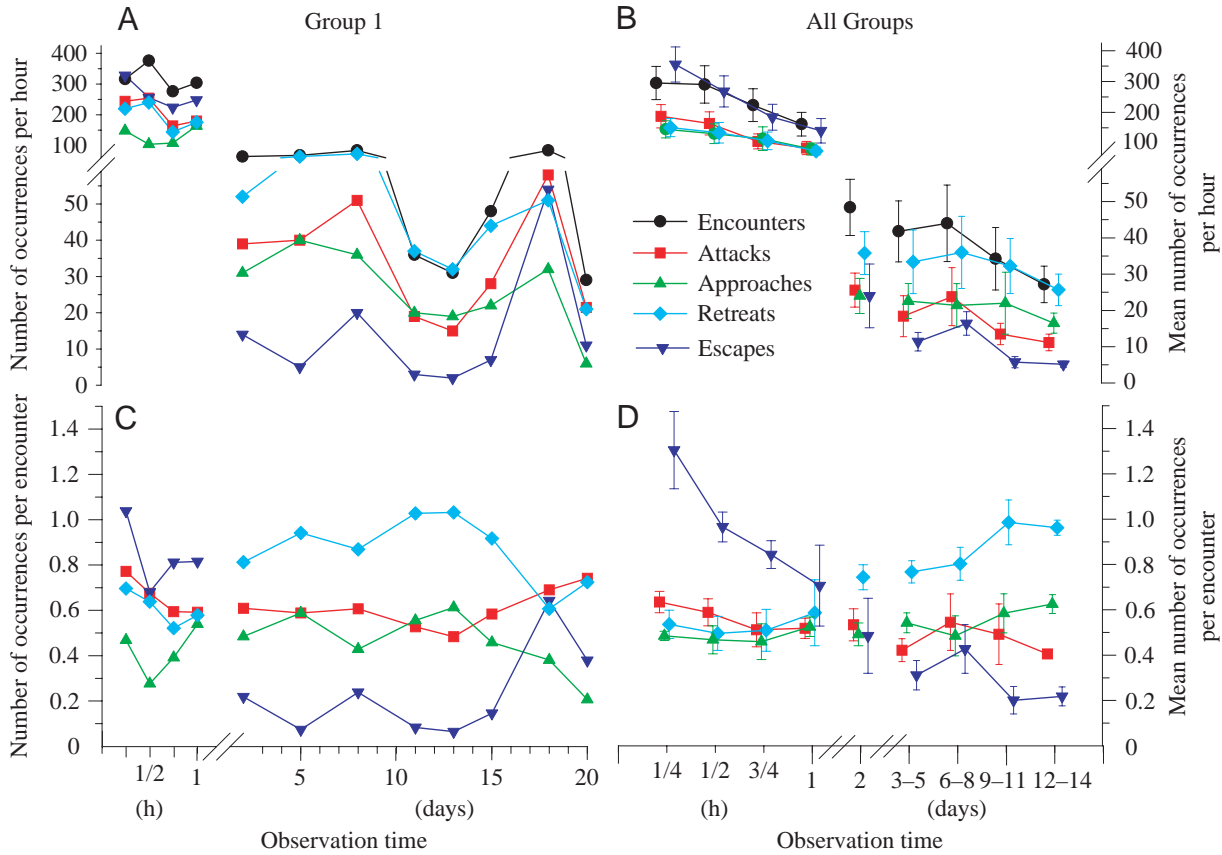


Fig. 3. Change in behavior during the formation of the dominance hierarchy. The frequencies (A,B) and proportions (C,D) of different types of behavior in group 1 (A,C) and in all groups (B,D) are plotted over a 2 week period following group formation. (A) The frequencies of encounters and of each type of behavior in group 1 are plotted as the number of displays of the behavior pattern by all members of the group per hour during each day of observation. The hour of observation on day 1 (to the left of the break on the time axis) is broken into four 15 min periods. (B) The frequencies of each behavior pattern were calculated similarly for all groups, and the means and standard error of the means (S.E.M.) of the groups are plotted for the four 15 min periods on the first day (to left of the first time axis break), for the second day (between time axis breaks) and for 3 day intervals thereafter. The pooling of data in 3 day intervals was necessitated by the different collection times for each group following the second day (see Materials and methods). In this analysis, the average frequency of each group was calculated for 3 day intervals, and these group averages were averaged to obtain the mean \pm S.E.M. over the groups ($N=5$). Each plot is slightly offset from the others along the time axis for clarity of display. (C) The number of occurrences per hour of each type of behavior of group 1 was divided by the corresponding rate of encounters to obtain the proportion of encounters in which each behavior pattern occurred. As each behavior pattern can occur more than once during an encounter, these ratios are occasionally larger than 1. (D) The corresponding proportions of each type of behavior over all groups. For each group, the mean number of occurrences of each behavior pattern per hour was divided by the average number of encounters per hour to obtain a behavioral proportion for each group. The mean values of these group proportions \pm S.E.M. ($N=5$) were plotted. The error bars represent the S.E.M. of those latter averages.

1 and 2, and a continued gradual decline over the next 2 weeks of observation. At the same time, the relative frequencies of several of the behavior patterns changed along the lines experienced in group 1 (Fig. 3D). The relative frequencies of attack and approach remained approximately constant, with approaches gradually replacing attacks over the entire period. The frequency of escapes declined nearly continuously, whereas that of retreats increased on day 2 and remained high thereafter.

Immediate formation of a superdominant dominance hierarchy

The vigorous aggressive activity of one animal immediately

upon formation of each group quickly led to the formation of a dominance hierarchy in which one animal was superdominant to the others. This is reflected in the cardinal dominance values that, when the necessary assumptions were satisfied by the data, were calculated for each animal in each group for the four 15 min intervals of the first hour of observation on day 1 and for each hour of observation on subsequent days (Boyd and Silk, 1983; see Materials and methods). These values, which were based on the pattern of wins and losses of all animals in the group during the observation period, show that such a 'superdominant' dominance hierarchy was well established in each group within the first 15 min (Fig. 4). In group 1, animal C had a relative

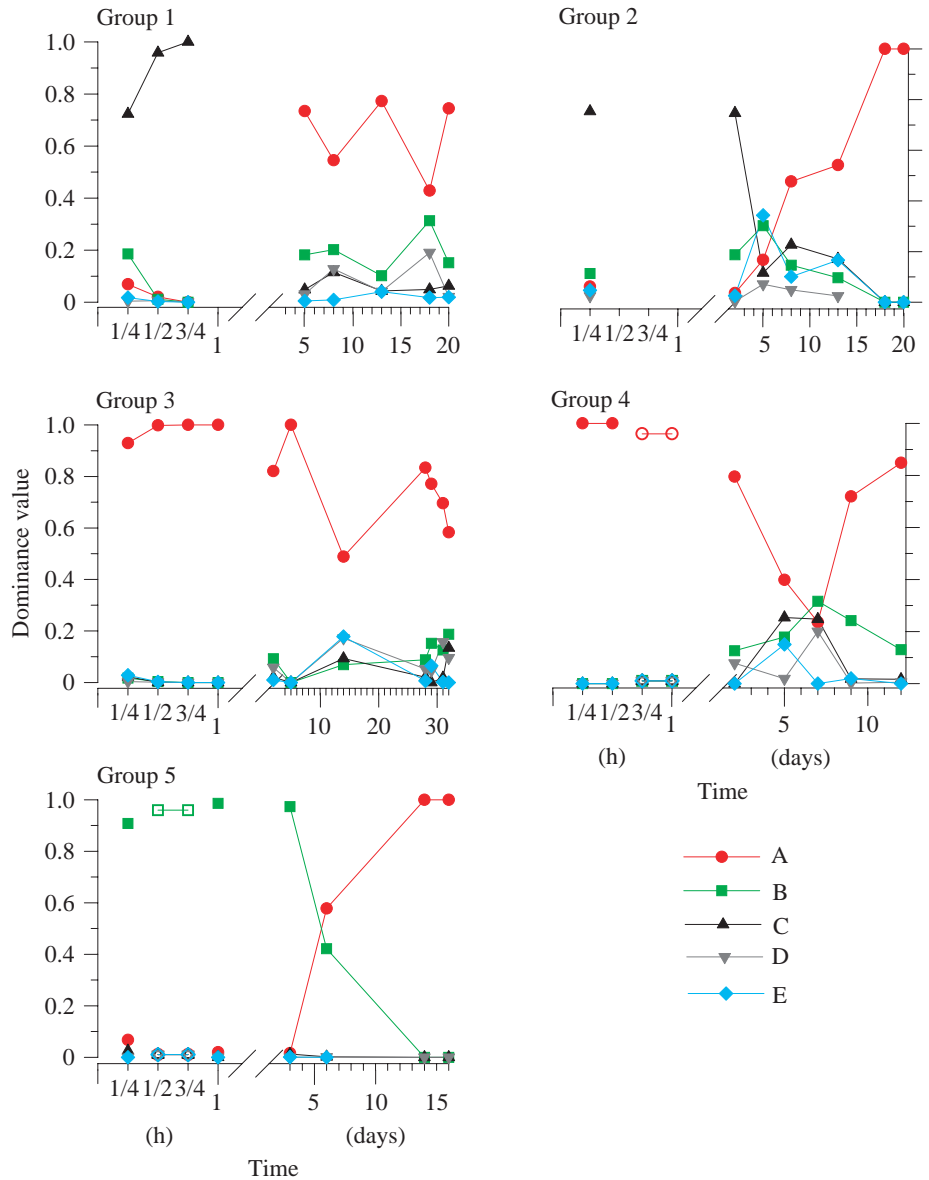


Fig. 4. Formation of superdominant hierarchies among juvenile crayfish. The cardinal dominance values of all the animals in each of the five groups are plotted over their periods of observation. The cardinal dominance values were calculated from the win/loss records of all the animals in the group over the period of observation according to the method of Boyd and Silk (1983; see Materials and methods). Days of observation differed among the groups (see Materials and methods). Values for some days when observations were made are missing because the hierarchy was circular or because one of the other assumptions of the method was violated (see Materials and methods). Open circles (day 1, groups 4 and 5) represent values calculated for one 30 min period rather than for the individual 15 min periods, which could not be calculated separately.

dominance value above 0.7 during the first 15 min, when the other animals were all rated below 0.2 (note that the sum of the cardinal dominance values of the five animals equals 1.0) (Fig. 4). During the subsequent 30 min, C won all but three of its encounters, so that its dominance value approached 1.0, and the values of the others approached zero. Values were not calculated for the last 15 min because the hierarchy became circular (see Materials and methods).

In four of the five groups, the superdominant hierarchy was maintained throughout the hour. In these groups, the level of agonistic activity was maintained at high levels, whereas in group 2, the number of attacks and wins by the initially dominant animal C fell over the final 45 min to the low levels of the other four. These low values, and the absence of wins by animal D during this period, made the dominance values indeterminate.

The domination of animal C in group 1 broke down on day 2, when all five animals had dominance values of 0.4 or less.

The rise to superdominance of animal A was apparent by day 5, when its dominance value exceeded 0.7, and values for the others were less than 0.2. This relationship was maintained throughout the rest of the experimental period, except on day 18, when the aggression of B caused the superdominant hierarchy to become a more linearly graded hierarchy.

Groups 2 and 5 also experienced a turnover of superdominants during the period of observation. In group 2, the midsized animal C regained its superdominant position on day 2, but was later replaced as superdominant by the largest animal, A. In group 5, the second largest animal, B, was also replaced by the largest, A. In group 4, the superdominant hierarchy broke down on days 5 and 7, but was re-established with the original superdominant, A, still in place. In group 1, animal A experienced a similar challenge on day 18 but recovered on day 20, whereas in group 3, the superdominant position of animal A was never seriously challenged during the 30 days of observation.

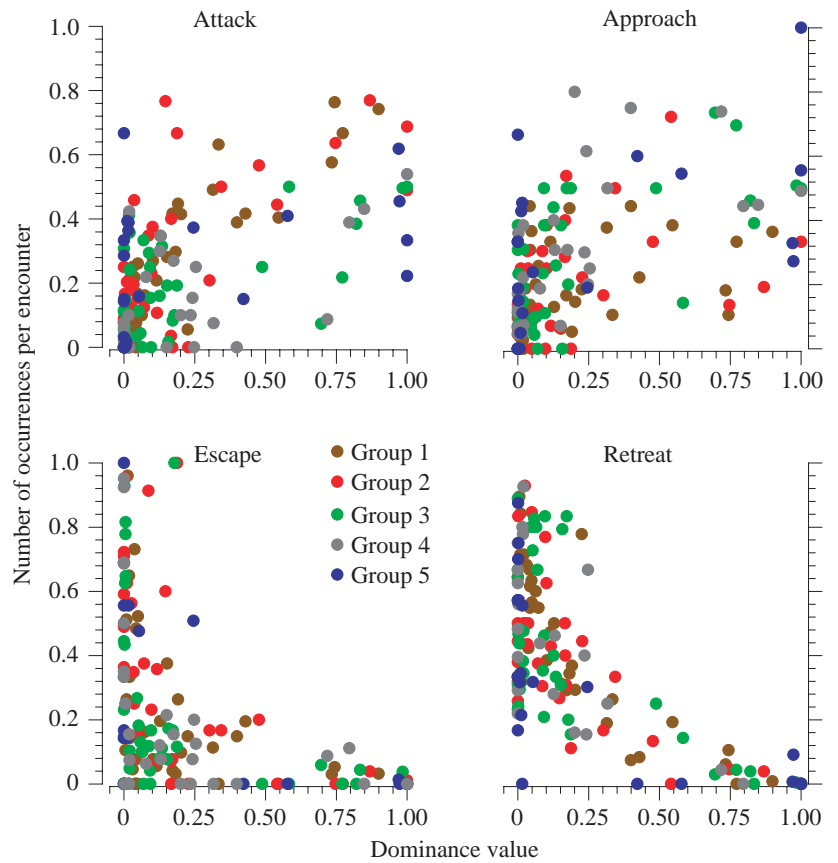


Fig. 5. Behavioral frequency and social dominance. The relative proportion of each behavior (attack, approach, retreat and escape), expressed as the number of occurrences per encounter during each hour of observation, is plotted against the cardinal dominance value of the animal during that hour for all groups, animals and days for which dominance values could be calculated. The points are color-coded according to group.

The dependence of different types of behaviors on cardinal dominance

Dominant animals in group 1 appear to have gained their position by being aggressive and displaying a higher frequency of attacks and approaches than their opponents (Figs 1, 2). Indeed, in group 1, 95% of encounters were won by animals that initiated an attack or approach, and in the other groups, the rate of wins followed the same pattern as the rate of attacks (Fig. 2). Analysis of the relationship between behavioral frequency and dominance in all five groups bears this out; attack and approach were used by more dominant animals during agonistic interactions, and retreat and escape were used by less dominant animals. This conclusion is supported by the data shown in Fig. 5, in which the relative proportion (i.e. the number of occurrences/the number of encounters) of each of the four types of behavior is plotted for each animal against the cardinal dominance value of the animal for the observations on that day. Attacks and approaches assume a much larger role in the overall behavior of an animal when its cardinal dominance value is 0.3 or above, whereas escape and retreat play a large role in animals with dominance values of 0.2 or below.

Discussion

Formation of a superdominant hierarchy

In four of the five groups, the high level of aggression displayed by one animal from the outset in each group led

immediately to the formation of a superdominant dominance hierarchy that persisted throughout the life of the group. Although the level of aggression was greatly reduced after the first day, the superdominant hierarchy persisted, although not always with the same superdominant individual. The superdominant made more attacks and approaches than the others, won those encounters, and was rarely attacked in return. On occasion, however, the superdominant hierarchy broke down. The initial superdominant was replaced on such occasions in three of the groups, and retained its position after breakdowns in the two other groups.

These results make it possible to identify several factors that may contribute to the formation and maintenance of a superdominant hierarchy. The first factor is the high initial level of aggressiveness displayed in four of the groups by one animal. This animal initiated most of the attacks and won most of its encounters on day 1, thereby establishing and maintaining a superdominant hierarchy in its group. Attacking or approaching first nearly always led to victory (Copp, 1986). In these four groups, high numbers of attacks produced high numbers of wins for this one animal, while the numbers of attacks and wins by others in each group fell to low levels (Fig. 2). In group 2, the initial superdominant position of animal C was not sustained, perhaps because the initial level of aggression displayed by that animal was not high (10 attacks during the first 15 min) and then declined to values that were comparable with those of the other members of the group.

The second factor is the experience of winning, which

appeared to reinforce the aggressiveness of the winner, and the experience of losing, which appeared to inhibit aggression in the losers. In the four groups that established the superdominant hierarchy on day 1, the difference between the number of attacks made by the superdominant and the sum of attacks made by the other four animals increased to a peak over the middle part of the hour and then declined slightly at the end of the hour (Fig. 2B). This was accompanied by the near elimination of attacks and approaches among the non-dominant crayfish. The relative increase in the number of attacks and wins by the superdominant and the absolute decline in attacks and wins by the subordinates suggests that a positive feedback relationship exists between the number or frequency of attacks and the number or frequency of wins. In such a relationship, as an animal wins more victories, it is more likely to attack, which will enable it to win more victories. Similarly, as an animal loses more frequently, it is less likely to attack and therefore more likely to lose in subsequent encounters. In the present case, such a positive feedback relationship may be tempered by the complete dominance of one animal and complete subordination of the others. There is little need to keep attacking once complete dominance is achieved (Fig. 4), which may explain why the frequency of attacks and wins begins to decline (Fig. 2) once this has occurred.

The third factor is the relative size of the animal, which has been frequently identified as the major determinant of dominance order in crayfish (Rutherford et al., 1995; Pavey and Fielder, 1996; Ranta and Linström, 1992; Figler et al., 1995a). This last factor, the difference in relative size of the animals, emerged here when the largest animal became the superdominant at the outset (two groups) or when the largest animal replaced another as superdominant (three groups). In the latter cases, the largest animal became the superdominant after the level of agonistic activity had declined considerably and the apparent inhibition of attacks and approaches by the subordinates had diminished. It would appear, then, that size is a constant factor that favors winning by the largest animal, but that it can be overcome by a high level of aggressive activity by others. However, when aggressive activity declined as winners and losers were identified, the suppression of the largest animal lessened, and its size helped produce victories in encounters with the current superdominant and led to its replacement. The positive feedback mechanisms described above should lead to rapid transitions between superdominant and subdominant status.

Changes in behavior during the formation of a dominance hierarchy

The first change in behavior was the immediate onset of a high level of aggression within the group. The high frequency of aggressive interactions began to decline within the first 30 min as winners and losers were identified (Söderbäck, 1991; Guiasu and Dunham, 1997; Ranta and Linström, 1992). This decline is apparent in three changes in the interactions between crayfish. First, the rate at which agonistic interactions occurred was reduced on the second day to approximately 15% of its

initial high level. Second, aggressive animals shifted from greater reliance on attack on the first day to a more balanced use of attack and approach, in which no contact occurred, on the second day. Third, defensive behavior shifted from a strong reliance on escape to a nearly exclusive use of retreat after the first day. These last two changes indicate that the vigor of aggressive interactions had subsided and that the subordinate animals were likely to avoid physical contact by retreating before it occurred. All these changes continued to develop during subsequent days.

The processes that govern these different changes in behavior appear to operate over several different time scales. At the short end of the time scale are the initial decision to become highly aggressive in the presence of a strange conspecific and the decision by one of the two animals to withdraw. All these occur very quickly, within a few seconds among the juveniles observed here, and over a somewhat longer period among adults (Huber et al., 1997). This decision to withdraw by one animal then begins a prolonged divergence in the way each member of the pair of animals behaves towards the other. Aggression is quickly suppressed in the defeated animal, and future fights are shorter. Fights are initiated almost entirely by the winner, and the loser moves to avoid the winner. A longer-term process then occurs as the level of aggressive activity declines in both animals. The frequency of attacks and approaches drops dramatically during the first 24 h and then more slowly over the next 2 weeks as the animals adapt to their different roles. Over this longer time scale, subordinate crayfish are increasingly able to avoid approaches of the dominant by retreating before physical contact occurs. If the overall decline in agonistic activity is seen as the result of habituation of each of the animals to the others and to the environment (Peeke et al., 1971), then it is not unusual for habituation to be governed by a rapid initial process and a slower subsequent process (Bruner and Kennedy, 1970; Krasne and Wine, 1977).

The novelty of the new environment, followed by habituation to it, are probably contributing factors to the initial high level and subsequent large fall in agonistic activity experienced by all groups of crayfish. Crayfish actively explore a new environment upon introduction, but spend much less time exploring it upon reintroduction 24 h later (Basil and Sandeman, 1999). The time spent exploring continues to decline upon subsequent reintroductions at 24 h intervals until, by day 4, the time spent exploring is less than half of that on the first day. If the time spent exploring can be used as a measure of arousal, then it is likely that the animals in the present experiments were highly aroused when placed together into a new environment and greatly habituated at the time of the second period of observation 24 h later. How much of the initial level of aggression can be attributed to the arousal induced by the novel environment, and how much of the subsequent decline can be attributed to habituation to that environment, remains to be determined.

Superdominant hierarchies among adult crayfish

Analysis of previously published data of wins and losses

among groups of four adult crayfish has shown that they, like the juveniles described above, display a superdominant organization, with one animal as superdominant and others clustered together well below the first. Cardinal dominance values were calculated from win/loss data from groups of four animals studied by Bovbjerg (1953), Lowe (1956) and Copp (1986). In the animals studied by Bovbjerg, the superdominant had a cardinal dominance value near 0.95, and values for the other three were all below 0.04. In one study by Lowe, the value for superdominant was near 0.8, that of the next was 0.18 and those of the remaining two were below 0.02. The animals studied by Copp were the most graded in their dominance. The value for most dominant was near 0.6, that of the next was at 0.25 and those of the final two were below 0.11. As with the juvenile crayfish studied here, the dominance order among at least one group of adult crayfish correlates well with size (Lowe, 1956).

Relationship between behavior and dominance

The relationship between dominance and the frequency of aggressive or defensive behavior is particularly clear in the juvenile crayfish. More dominant animals are much more likely to attack or approach, and less dominant animals are much more likely to escape or retreat. As described above, this relationship creates the positive feedback mechanism that leads to the rapid formation or reformation of the superdominant dominance hierarchy. Although this relationship has found support in other studies (Copp, 1986; Ranta and Linstrom, 1992), it has not been universally observed among crayfish. Bovbjerg (1953) found no difference in the frequencies of aggressive and defensive behavior between animals of different dominance rank. This is surprising given the highly skewed nature of the hierarchy created by the animals in his study. Also surprising is the report that in encounters between members of one species, *Cambarus robustus* Girard, the eventual losers initiated the vast majority (81.5%) of the very first fights in the overall agonistic contests (Guisu and Dunham, 1997). Here, the prospective losers initiate the fight with an 'ambivalent approach', and so reveal their lack of aggression.

Physiological correlates of changes in behavior

The physiological substrates of these processes are not yet identified, but correlated phenomena have been observed. An injection of serotonin into subordinate crayfish has been found to prevent withdrawal from a fight (Huber et al., 1997). The effect of serotonin on inhibition of withdrawal from a fight can be blocked by co-administration of the serotonin uptake inhibitor fluoxetine, suggesting that the blockage of withdrawal is mediated by the release of supranormal amounts of serotonin from serotonergic neurons that had taken up the injected drug (Huber et al., 1997; Fuller, 1996). These experiments also make clear that serotonin does not directly promote aggression. The implication is that these short-term changes in behavior, including both the release of aggression and the decision to withdraw, result from the release of two or more substances

that condition the nervous system in specific ways. In mammals, corticosteroids and arginine vasopressin together with serotonin have been implicated in the mediation of changes in social rank. For example, both corticotropin-releasing factor and arginine vasopressin mRNA levels are reduced in newly subordinate rats (Albeck et al., 1997).

The longer time-scale effects, including the gradual reduction in aggressiveness and the avoidance of the dominant by subordinates, may result in part from changes in patterns of receptor distribution. The effect of serotonin on the stimulus threshold for tailflip escape changes in opposing fashions in newly subordinate and dominant crayfish over a 2 week period following their pairing (Yeh et al., 1996, 1997). These changes appear to result from changes in the balance of serotonin receptors on the command neuron for escape, such that inhibitory receptors come to outweigh facilitatory receptors on the command neuron in the new subordinate, and the reverse occurs in the new dominant. This mechanism may contribute to the steadily decreasing use of tailflip escape in new subordinates over the 2 week period of hierarchy formation. Experiments in free-acting animals indicate that the stimulus threshold for command-neuron-evoked escapes shows a large increase in subordinates during fighting and a small increase in dominants (Krasne et al., 1997). Serotonin released during fighting may contribute to these changes in both animals. The small increase in stimulus threshold in dominants is counter to the decrease expected from the observed effects of applied serotonin in dominants (Yeh et al., 1997), and suggests that a more complex mechanism is involved.

Context of these results

The conditions of this study were chosen to increase the number of encounters and to make dominance depend on pairwise interactions rather than on the ability to retain some desired resource, such as a shelter. Prolonged isolation and arousal caused by hand transfer of the animals to the experimental aquarium may have contributed to the initial high level of aggressiveness by one animal. The lack of a shelter removed an object of contention and a way to compartmentalize the shared space. If a shelter were present, the dominant animal would probably occupy it and would not, therefore, engage in the high level of attacks on subordinates seen here. As a result, the dynamics of the agonistic interactions, if not the superdominant organization of the group, would probably be different. Another special condition is that the groups chosen for study under these conditions were those in which all the animals survived for an extended period. Juvenile crayfish are cannibalistic, especially when crowded, and some groups suffered loss of members shortly after formation. Two of the groups studied here experienced the loss of a member; group 2 lost the next to smallest animal, D, after day 15, and group 5 lost the smallest animal after day 6. In both instances following the loss of a member, the dominance value of the superdominant, A, increased to near 1 and the dominance values of the remaining four animals dropped to near 0 (Fig. 4).

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